vol. 187, no. 3 the american naturalist march 2016

Beyond Thermal Performance Curves: Modeling Time-Dependent Effects of Thermal Stress on Ectotherm Growth Rates

Joel G. Kingsolver[1,*] and H. Arthur Woods[2]

1. Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599; 2. Division of Biological Sciences, University of Montana, Missoula, Montana 59812

Submitted May 29, 2015; Accepted September 14, 2015; Electronically published January 14, 2016

Online enhancement: supplementary material. *Dryad data:* http://dx.doi.org/10.5061/dryad.1017n.

abstract: Thermal performance curves have been widely used to model the ecological responses of ectotherms to variable thermal environments and climate change. Such models ignore the effects of time dependence—the temporal pattern and duration of temperature exposure—on performance. We developed and solved a simple mathematical model for growth rate of ectotherms, combining thermal performance curves for ingestion rate with the temporal dynamics of gene expression and protein production in response to high temperatures to predict temporal patterns of growth rate in constant and diurnally fluctuating temperatures. We used the model to explore the effects of heat shock proteins on larval growth rates of *Manduca sexta*. The model correctly captures two empirical patterns for larval growth rate: first, maximal growth rate and optimal temperature decline with increasing duration of temperature exposure; second, mean growth rates decline with time in diurnally fluctuating temperatures at higher mean temperatures. These qualitative results apply broadly to cases where proteins or other molecules produced in response to high temperatures reduce growth rates. We discuss some of the critical assumptions and predictions of the model and suggest potential extensions and alternatives. Incorporating time-dependent effects will be essential for making more realistic predictions about the physiological and ecological consequences of temperature fluctuations and climate change.

Keywords: ectotherm, fluctuating environments, temperature, heat stress, climate change, growth rate.

Introduction

Most organisms experience variation in temperature and other abiotic variables at a variety of temporal and spatial scales. Many ectotherms adapt to local conditions via differences in their thermal sensitivity or thermal performance curves (Huey and Stevenson 1979; Huey and Kingsolver 1989). For example, populations may differ in optimal temperature, thermal breadth, and cold and heat tolerance (Angilletta 2009; Sunday et al. 2011). Thermal performance curves are widely used in models for the biological responses of ectotherms to recent and future climate change (Deutsch et al. 2008; Huey et al. 2009; Sinervo et al. 2010; Kingsolver et al. 2013; Vasseur et al. 2014), including changes in mean temperatures, diurnal and seasonal cycles, and climatic extremes around the globe (Intergovernmental Panel on Climate Change 2007; Williams and Jackson 2007; Williams et al. 2007; Battisti and Naylor 2009). Because thermal performance curves for rates of growth, fitness, and other biological processes are strongly nonlinear, patterns of variation in temperature can have important effects on mean and variance in performance and fitness (Martin and Huey 2008; Angilletta 2009; Kingsolver et al. 2009; Vasseur et al. 2014). In particular, the mean performance in a given thermal environment generally cannot be estimated using that environment's mean temperature (Martin and Huey 2008).

Thermal performance curves implicitly assume that performance depends only on current temperature—and not on past exposure, including length of time at the current temperature or the pattern of exposure to other, different temperatures in the past. However, many recent studies have shown that the duration of exposure influences performance even at nonlethal temperatures and that the upper thermal range for performance decreases with increasing duration of exposure (Schulte et al. 2011; Mislan et al. 2014; Rezende et al. 2014). For example, in insect eggs and larvae, temperatures that maximize performance (development and growth rates) at short timescales (4–24 h) can be suboptimal or even lethal over longer timescales (Reynolds and Nottingham 1985; Kingsolver and Woods 1997; Kingsolver 2000; Petersen et al. 2000; Kingsolver et al. 2004; Woods and Bonnecaze 2006; Kingsolver and Nagle 2007; Potter et al. 2009). Several recent studies demonstrate that thermal performance curves based on data from constant temperature experiments yield

* Corresponding author; e-mail: jgking@bio.unc.edu.

Am. Nat. 2016. Vol. 187, pp. 000–000.
0003-0147/2016/18703-56300$15.00.
DOI: 10.1086/684786

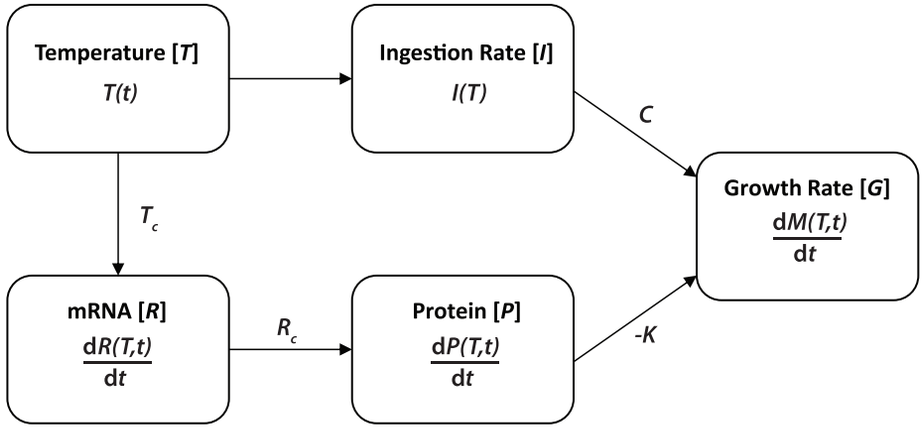


Figure 1: Diagram of the main components of the model. See text and equations (1)–(4).

poor predictions about mean rates of growth and development under diurnally fluctuating conditions (Kingsolver and Nagle 2007; Kingsolver et al. 2009, 2015; Niehaus et al. 2012). These results call into question the common practice of using thermal performance curves measured at constant temperatures to predict responses of ectotherms to diurnal fluctuations and to climate change (Deutsch et al. 2008; Sinervo et al. 2010; Kingsolver et al. 2013; Vasseur et al. 2014).

The effects of duration and pattern of thermal exposure—which we refer to here as "time-dependent effects"—can affect performance negatively or positively. Negative effects generally stem from repeated acute heat or cold shocks, which can be stressful and are known to reduce subsequent performance and survival in many ectotherms (Feder and Hofmann 1999). For example, organisms that live in highly variable thermal environments, as do many terrestrial insects and intertidal invertebrates, frequently induce heat shock proteins (HSPs) within the range of body temperatures normally experienced in the field (Hofmann 1999; Dahlhoff and Rank 2007; Tomanek 2010). While these responses can improve short-term survival, continued overexpression of HSPs can negatively affect rates of metabolism, growth, development, and fertility (Sorensen et al. 2003; Tomanek 2010).

Heat shocks can also induce changes in gene expression and protein synthesis in a variety of other genes (Sorensen et al. 2005; Porcelli et al. 2015). Recent RNA-seq studies in a range of animal species show that heat shocks can alter patterns of expression of genes involved in metabolism, antioxidant production, and ion transport (Meyer et al. 2011; Schoville et al. 2012; Barshis et al. 2013; Smith et al. 2013; Gleason and Burton 2015; Seneca and Palumbi 2015). Upregulation of genes and protein production beyond HSPs can reduce subsequent growth and survival, but quantitative estimates of these effects are largely lacking.

Positive effects can also stem from acute exposure to high temperatures, depending on temperatures subsequently experienced by the organism. In particular, brief exposure to high but sublethal temperatures may induce HSPs and increase survival when exposed to subsequent heat shocks, a form of adaptive acclimation called heat hardening (Lindquist and Craig 1988; Kregel 2002). Despite the importance of these time-dependent effects, we lack a quantitative framework for integrating them into studies using thermal performance curves. Such integration is necessary if we are to understand the long-term performance of ectotherms in fluctuating thermal environments.

Here we move beyond thermal performance curves by developing a simple mathematical model for growth rate of ectotherms in changing thermal environments. The model integrates thermal performance curves for ingestion rate with the temporal dynamics of gene expression and proteins produced in response to stressfully high temperatures to predict temporal patterns of growth rate in constant and diurnally fluctuating temperatures. We illustrate the model using information on ingestion, HSPs, and larval growth rates of *Manduca sexta*, but the general features of the model should apply broadly to other ectotherms, proteins, and processes. Despite its simplicity, the model correctly captures two qualitative empirical patterns for larval growth rate: the influence of timescale on the shape of thermal performance curves and the consequences of diurnal fluctuations for mean growth rate. We discuss some of the critical assumptions and predictions of the model and suggest potential extensions and alternatives.

The Model

Overview

Our strategy is to extend a simple model of a thermal performance curve for an ectothermic animal so that it incorporates time-dependent effects on growth rate. Time-

dependent effects are added by modeling the effects of stressfully high temperatures on stress proteins. The basic elements of the model are shown in figure 1. Suppose that temperature T varies as a function of time t: $T(t)$. First, temperature determines ingestion rate, represented by a thermal performance curve: $I(T)$. Second, temperatures above some critical temperature (T_c) induce the expression of mRNA for a stress gene (or genes). The rate of change in mRNA concentration, dR/dt, depends on both temperature and time. Third, mRNA levels above some critical level (R_c) cause production of a stress protein (or proteins) P; the rate of change in protein concentration, dP/dt, depends on both temperature and time. Fourth, growth rate—the rate of change of body size, dM/dt—is determined by two factors: ingestion rate $I(T)$ (and some conversion efficiency, C) and the cost of the stress protein (weighted by a cost parameter, K). As a result of these processes, growth rate is influenced by both temperature and time.

In "Discussion," we consider possible extensions to and alterations of this basic model structure. In the next section, we specify it in a particular mathematical form, in which the temporal dynamics of mRNA and protein concentrations are represented by first-order rate processes.

Model Development

The model above (fig. 1) describes a system of three ordinary differential equations (ODEs) for changes in mRNA level (dR/dt), protein level (dP/dt), and mass (dM/dt). Temporal changes in temperature $T(t)$ represent an input (forcing) function to this system of ODEs. Here we describe each component of the model.

Let $I(T)$ be the thermal performance curve for ingestion rate. Here we use a modified Gaussian function, which describes thermal performance curves for rates of feeding, growth, fitness, and locomotion in many ectotherms (Frazier et al. 2006):

$$I(T) = I_m e^{-e[\rho(T-T_{opt})-6]-\sigma(T-T_{opt})^2}. \quad (1)$$

Here T_{opt} is optimal temperature, I_m is maximal ingestion rate at T_{opt}, and ρ and σ and are the widths of the performance curve below and above T_{opt}, respectively (fig. 2*A*).

Let $R(T, t)$ = mRNA concentration, where the rate of change in R depends on T, with a first-order temporal response:

$$\frac{dR}{dt} = -\left[\frac{t}{\tau_R}\right](R - R_f), \quad (2a)$$

where R_f is the equilibrium (final) level of expression and τ_R is the time constant for changes in mRNA concentration. Note that dR/dt is negative when $R > R_f$ and is positive when $R < R_f$. We model R_f as a sigmoid function of temperature T:

$$R_f = \frac{R_m}{\{1 + e^{-a_R(T-T_c)}\}}, \quad (2b)$$

where T_c is the critical midpoint temperature for gene expression and R_m is maximal expression level. Note that

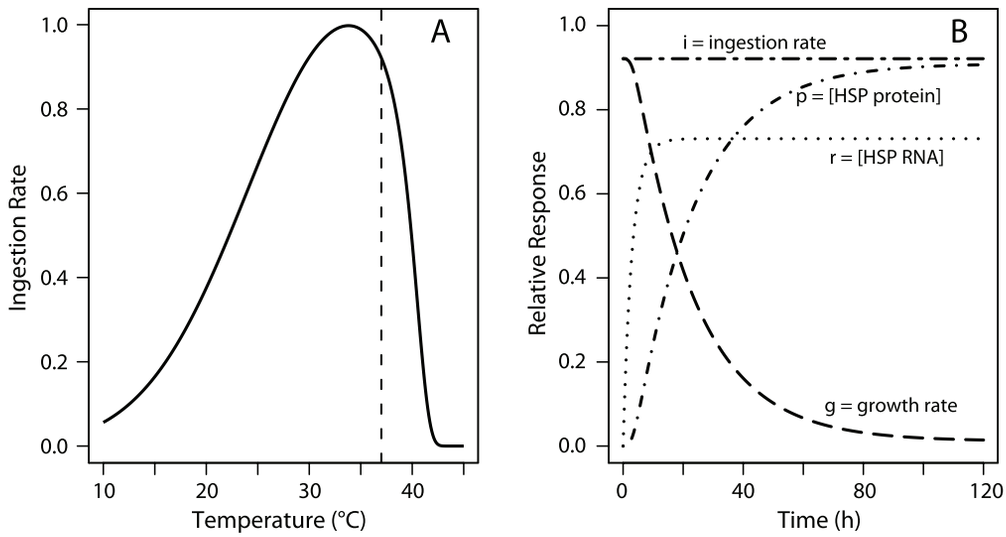


Figure 2: Model predictions for a step change in temperature at time $t = 0$ to a constant temperature of $T_0 = 37^\circ$C. *A*, Thermal performance curve for ingestion rate (i) as a function of temperature; $T_0 = 37^\circ$C (dashed vertical line) is indicated. *B*, Relative ingestion rate (i), mRNA concentration (r), protein concentration (p), and growth rate (g) as functions of time. HSP = heat shock protein.

$R_f \sim 0$ for $T \ll T_c$ and that $R_f \sim R_m$ for $T \gg T_c$; T_c is the midpoint of the switch (at rate a_R) from zero to maximum equilibrium level of mRNA.

Let $P(T, t) =$ protein concentration, where the rate of change in P depends on T, with a first-order temporal response:

$$\frac{dP}{dt} = -\left[\frac{t}{\tau_P}\right](P - P_f), \tag{3a}$$

where P_f is the equilibrium (final) protein concentration and τ_P is the time constant for changes in protein concentration. We model P_f as a sigmoid function of RNA R:

$$P_f = \frac{P_m}{\{1 + e^{-a_P(R-R_c)}\}}, \tag{3b}$$

where R_c is the critical midpoint level of mRNA for protein production and P_m is the maximum protein concentration.

Growth rate, $G(T, t) = dM/dt$, reflects both ingestion and the cost of stress proteins. Thus, we have

$$G(T, t) = \frac{dM}{dt} = CI(T) - KP(T, t), \tag{4}$$

where C is the conversion efficiency from ingestion to growth and K is cost to growth due to the production of HSPs and their negative effects on other biological processes. Note that in this model the cost is proportional to protein level P. In the language of systems biology, the equations above represent a proportional control model (see "Discussion").

Inspection of equations (1)–(4) suggests a natural way to rewrite them in dimensionless form. We define

$$i = \frac{I}{I_m}, \tag{5a}$$

$$r = \frac{R}{R_m}, \tag{5b}$$

$$p = \frac{P}{P_m}, \tag{5c}$$

$$g = \frac{G}{CI_m}, \tag{5d}$$

where CI_m represents the maximal growth rate at optimal temperature T_{opt} in the absence of costs of P. Thus, we present all results in this dimensionless form. We present the full model in this form in appendix A.

For simplicity, the model above (eqq. [1]–[5]) assumes that growth rate (rate of mass increase) is independent of mass. Including size scaling of growth does not alter the qualitative results of the model, but such scaling will compound the consequences of time-dependent effects for growth rate and size. A model incorporating allometric scaling for consumption and growth is presented in appendix B.

Parameters

We illustrate the model by considering feeding, growth, and HSP responses of herbivorous larvae of the tobacco hornworm, *Manduca sexta*, for which data are available that can be used to estimate some of the model parameters. Parameters for the ingestion rate function $I(T)$ (eq. [1]) and for conversion efficiency C (eq. [4]) were estimated from data on short-term (4-h) ingestion and growth rates for fifth-instar larvae of *M. sexta* (Kingsolver and Woods 1997). These data indicate an optimal temperature for ingestion of ~34°C (fig. 2) when reared at 25°C. Brief heat shocks (2 h) at 38°C and above can induce both gene expression and synthesis of HSPs in *M. sexta* (Fittinghoff and Riddiford 1990), and under diurnally fluctuating rearing conditions daily maximum temperatures above 30°–35°C may induce heat shock responses (J. G. Kingsolver, H. MacLean, J. K. Higgins, K. E. Augustine, and S. B. Goddin, unpublished data). As a result, we let $T_c = 35°C$ (eqq. [2]) in our analyses. There are few data relevant to establishing values for R_m, R_c, P_m, a_R, and a_P (eqq. [2], [3]); for our analyses, we let $R_c = 0.5R_m$, $P_m = 1$, $a_R = 0.5$, and $a_P = 1$. Note that in the nondimensional form of the model (eqq. [5], appendix A, and "Model Results") many of these parameters are eliminated. The time constants τ_R and τ_P, which reflect the response times of mRNA expression and protein production, respectively, play key roles in determining the temporal dynamics of the model. Heat shock studies in many aquatic and terrestrial ectotherms show responses of heat shock gene expression on timescales of 1–2 h (Feder and Hofmann 1999; Hofmann 1999; Sorensen et al. 2003; Tomanek 2010). In contrast, the timescales of HSP responses vary from hours to days in different insect species (Fittinghoff and Riddiford 1990; Bahrndorff et al. 2009; Karl et al. 2012). In our simulations, we let $\tau_R = 2$ and $\tau_P = 20$ but explore other values and their consequences in the supplementary material, available online (see figs. S2, S3 and "Discussion"). Finally, we let the proportional cost of protein concentration P for growth rate (K) be $K = 0.4$, but we consider other values in the supplementary material (fig. S1). As long as $K > 0$, its value does not alter the qualitative results of the model.

The model (eqq. [1]–[5]) consists of a set of nonlinear ODEs, which we solved numerically in R using the package deSolve (Soetaert et al. 2010). R code for running the model is available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.1017n (Kingsolver and Woods 2016).

Model Results

Step Changes in Temperature

Consider the simple case in which relative expression (r) and protein (p) levels are initially 0 and there is a step change to a new temperature T_0 at time $t = 0$. For illustration, consider a thermal performance curve for ingestion rate (fig. 2*A*), and let $T_c = 35^\circ$C and $T_0 = 37^\circ$C. Ingestion rate i at this temperature is relatively high and constant (fig. 2*B*). Because T_0 exceeds the midpoint temperature T_c, expression level r rises rapidly to approach an equilibrium level within 20 h. Protein level p increases more slowly, approaching a high equilibrium value after 80–100 h. Because of the costs associated with protein concentration, growth rate g declines from an initially high level, approaching 0 after 80–100 h. The rate of decline in growth reflects both the cost of protein concentration and the time constant associated with protein synthesis. The rate and magnitude of the decline in growth rate g is determined by cost K. When $K = 0$ (no cost), growth rate g is constant and is simply determined by the ingestion rate I; when $K = 0.5$, growth rate becomes negative after ~40 h (fig. S1 in the supplementary material).

These patterns depend strongly on the initial temperature T_0 in relation to the midpoint temperature T_c. Suppose we consider a range of initial temperatures from 15° to 40°C (fig. 3). For temperatures between 15° and 30°C, growth rate (and ingestion rate; see fig. 2*A*) increases with increasing temperature and remains relatively constant over time (fig. 3*A*). At 35°C, growth rate is initially high, reflecting the high ingestion rate at this temperature, but declines by more than 50% over 60–100 h as protein levels increase. This decline occurs even though $T_0 < T_c$ because T_c represents the inflection point of a smooth sigmoidal curve—that is, there still is some production of r and p below T_c. At 40°C, growth rate is initially intermediate but rapidly declines to negative values within 18 h. As a result of these patterns, the thermal performance curve for growth rate varies with time. To illustrate this, we plot growth rate g as a function of temperature at different time points from 0 to 80 h (fig. 3*B*). Initially, growth rates are highest at temperatures near the optimal temperature for ingestion (34°C), but growth rates decline rapidly with time for temperatures above ~32°C. After 48 h, growth rates are highest at temperatures near 30°C, and growth rates are negative at temperatures above 37°–38°C. Thus, both optimal and maximal temperatures for growth decline with time as a result of the costs and temporal dynamics of protein concentration.

Diurnal Fluctuations in Temperature

In the more general case where temperatures fluctuate diurnally, RNA concentration r, protein concentration p, ingestion rate i, and growth rate g all vary with time. We consider the situation where temperature varies daily between 20° and 40°C, with a mean temperature of 30°C (fig. 4*A*). Maximal ingestion rates occur in the morning and afternoon each day, as temperatures approach the optimal temperature for ingestion (fig. 2*A*), but decline to lower rates both at midday and at night when temperatures are far from optimal (fig. 4*B*). As temperatures move above and below

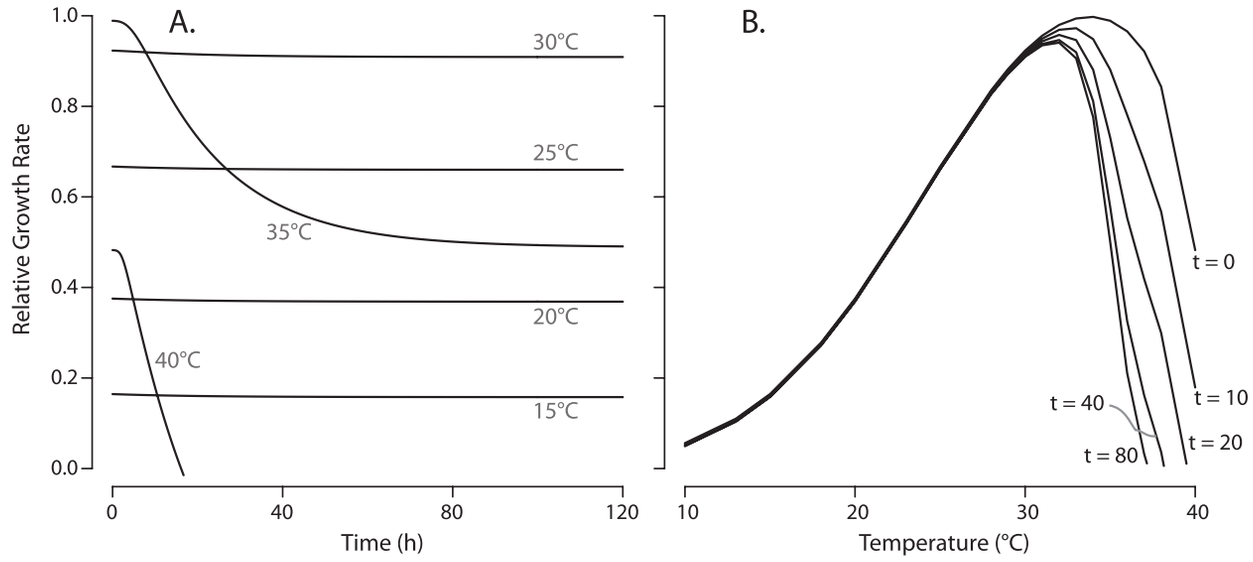


Figure 3: Model predictions of relative growth rate for step changes to different constant temperatures (T_0) between 15° and 40°C. *A*, Relative growth rates as functions of time for different T_0: 15°, 20°, 25°, 30°, 35°, and 40°C. *B*, Relative growth rates as functions of temperature at different times: $t = 0$, 10, 20, 40, and 80 h.

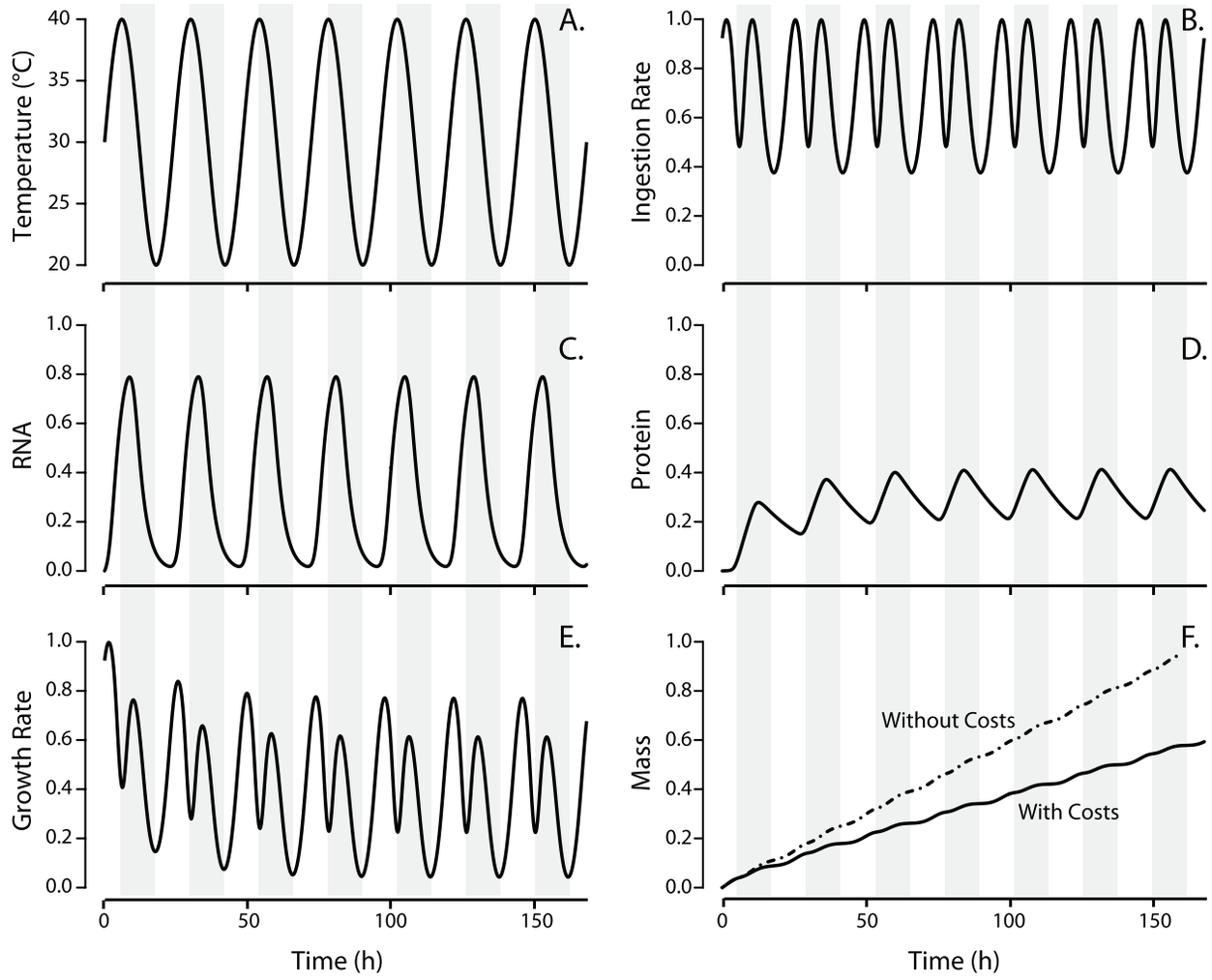


Figure 4: Model predictions for changes over time under diurnally fluctuating temperature conditions. *A*, Temperature. *B*, Relative ingestion rate. *C*, Relative mRNA concentration. *D*, Relative protein concentration. *E*, Relative growth rate. *F*, Relative mass, with (solid line) and without (dotted line) costs of protein.

T_c each day, RNA levels increase quickly during the day and decline to near 0 at night (fig. 4*C*). Protein levels also increase and oscillate diurnally between $p = 0.2$ and $p = 0.4$ after 60 h (fig. 4*D*). Growth rates show a more complex diurnal pattern as a result of the diurnal fluctuations in ingestion rate, and they also decline over the first 0–80 h as a result of increased protein concentration (fig. 4*E*). To illustrate the consequences of this for mean growth over time (fig. 4*F*), we compare cumulative growth when there is a cost of protein (black line) to cost-free growth (dotted line). After 120 h, final mass is reduced by more than 40% due to the costs of protein level in these fluctuating temperature conditions.

The temporal dynamics of r, p, and g in diurnally fluctuating temperatures depend on the time constants associated with gene expression and protein production (eqq. [2a], [3a]). Changes in these time constants alter the temporal dynamics. Longer time constants reduce overall costs to growth, because protein levels do not rise as rapidly to very high levels. Nevertheless, the qualitative outcome—a reduction in growth rate and size over time—remains the same (fig. 4; figs. S2, S3 in the supplementary material). Note that, in response to simple sinusoidal diurnal fluctuations in temperature, r, p, and g eventually converge to a fixed diurnal pattern of change (see "Discussion").

Mean Growth Rates in Constant and Fluctuating Temperatures

To explore how nonlinear and time-dependent effects influence mean growth rates, we modeled growth in nine differ-

ent temperature scenarios, with three mean temperatures (20°, 25°, and 30°C) and three diurnal fluctuations (±0°, ±5°, and ±10°C). We computed final mass at the end of 14 days as a measure of mean growth rate during this period. We modeled growth rate both with ($K = 0.4$) and without ($K = 0$) costs of protein level (eq. [4]). When protein costs are included (fig. 5*A*), mean growth rate at higher mean temperatures declines strongly with increasing diurnal fluctuations. In the absence of costs (fig. 5*B*), increasing fluctuations increase mean growth rate at low mean temperatures but have the opposite effect at high mean temperature. This pattern results from the changes in curvature of the ingestion rate function with temperature (fig. 2*A*). Note that in the absence of costs, growth rate always increases with increasing mean temperatures over this temperature range, even for large diurnal fluctuations (fig. 3*B*). In contrast, when costs are present and there are large diurnal fluctuations, mean growth rate declines as mean temperature increases from 25° to 30°C (fig. 5*A*). As a result, large diurnal fluctuations reduce the mean growth rate by more than 50% at high mean temperatures. This suggests that time-dependent heat shock mechanisms can amplify the negative effects of diurnal fluctuations at higher temperatures.

Discussion

Consequences of Time Dependence

Numerous studies have documented the deleterious effects of exposure to high but nonlethal temperatures for growth, development, and other aspects of performance in ectotherms (Feder and Hofmann 1999; Sorensen et al. 2003; Tomanek 2010). Our goal was to develop a simple model that extends thermal performance curves to include the time-dependent effects of stress on growth rates in fluctuating thermal environments (Schulte et al. 2011). The simple model we propose successfully captures two important empirical consequences of time-dependent effects. First, at high temperatures initial growth rates can be rapid but may decline with continued exposure to these temperatures (figs. 2*B*, 3*A*). As a result, the shape of the thermal performance curve for growth rate may change with the duration of exposure to (constant) temperatures (fig. 3*B*). In particular, the optimal temperature, maximal temperature, upper thermal limits, and thermal breadth for performance all decline with increasing duration of exposure. This pattern has been reported for thermal performance curves of insect growth rates (Reynolds and Nottingham 1985; Kingsolver and Woods 1997; Kingsolver 2000; Petersen et al. 2000; Kingsolver et al. 2004; Woods and Bonnecaze 2006; Potter et al. 2009) and other aspects of ectotherm performance (Rezende et al. 2014). In our model, this pattern results from the time-dependent costs of proteins at higher temperatures.

Second, diurnal temperature fluctuations can reduce mean rates of growth and development to a greater extent than expected from the nonlinearity of thermal performance curves alone (e.g., fig. 2*A*). As a result, thermal performance curves measured in constant temperature conditions can give inaccurate predictions of mean rates of growth and development in fluctuating conditions (Kingsolver and Nagle

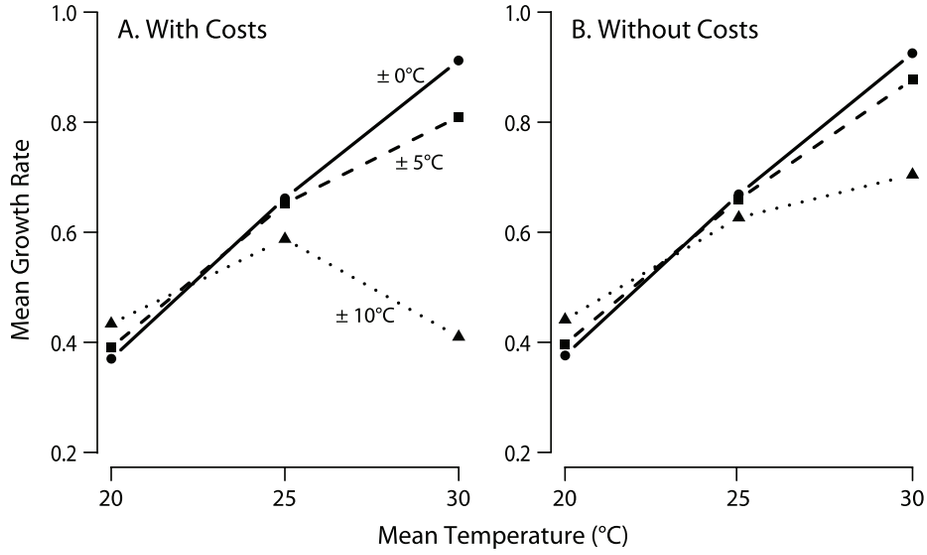


Figure 5: Model predictions of mean relative growth rate over 14 days as functions of mean temperature, for different amplitudes of diurnal fluctuations: (±0°C [circles, solid lines], ±5°C [squares, dashed lines], and ±10°C [triangles, dotted lines]). *A*, With costs of protein concentration ($K = 0.4$). *B*, Without costs of protein concentration ($K = 0$).

2007; Kingsolver et al. 2009, 2015; Niehaus et al. 2012). Our model correctly predicts that at higher mean temperatures diurnal fluctuations reduce mean growth rates as a result of the time-dependent effects of stress (figs. 4*F*, 5). In our model, reductions in growth rate occur as a result of increased levels of HSPs due to daily exposure to temperatures high enough to induce expression of heat shock genes and synthesis of the proteins they encode (fig. 4*C*, 4*D*). Such time-dependent effects call into question the common practice of using single, static thermal performance curves to predict responses of ectotherms to diurnal fluctuations and to climate change (Deutsch et al. 2008; Sinervo et al. 2010; Kingsolver et al. 2013; Vasseur et al. 2014). Model predictions based only on such static curves will become increasingly problematic as mean and maximum temperatures increase with climate change (Battisti and Naylor 2009). We suggest that understanding dynamic changes in thermal performance curves should be a high priority for future work (Schulte et al. 2011). It is possible that time-dependent effects at the upper end of thermal performance curves will have disproportionately strong effects on predictions about physiological and ecological responses of ectotherms.

These findings are also relevant to our interpretation of performance curves measured at different timescales. When performance curves are estimated by measuring performance at constant temperatures over many days (or over the entire life cycle, in the case of fitness), the measured values of performance will reflect costs due to time-dependent effects, and the resulting performance curve will underpredict mean performance in diurnally fluctuating temperatures. Conversely, when short-term (e.g., hourly) measurements are used to measure performance, the measured values of performance will not reflect costs due to time-dependent effects, and the resulting performance curve will overpredict mean performance in diurnally fluctuating temperatures (Kingsolver et al. 2015). Stochastic variation in the amplitude of diurnal fluctuations will likely exacerbate these effects.

Assumptions, Extensions, and Alternatives to the Model

Our model (fig. 1) assumes a few main elements based on our understanding of stress responses of ectotherms to high or fluctuating body temperatures (fig. 1): temperature affects ingestion rate in a nonlinear manner (fig. 2); higher temperatures can induce expression of genes associated with stress, leading to the production of stress proteins; and high levels of these proteins can reduce growth rate. The basic structure of this model should readily apply to other life stages (e.g., development rates in eggs or pupae) and other ecotherms (including microbes and plants).

A key determinant of the model predictions is the timescales of response for mRNA and protein concentration, as reflected in the time constants τ_R and τ_P, respectively (see eqq. [2], [3]). An important assumption in our model is that the time constants are the same for both production and degradation (i.e., for increases and decreases in R and P). Different dynamics for the two processes would alter the temporal dynamics of mRNA, protein, and growth rate. Including more detailed and realistic dynamics for these processes would allow better quantitative predictions.

We illustrated the model in terms of HSPs because our current understanding of these proteins in insects suggests that they play key, time-dependent roles in insect physiology and growth. Exposure to high temperatures can induce HSPs and other stress responses (Feder and Hofmann 1999). Although HSPs may increase survival during subsequent heat shocks, chronically high levels of HSPs due to sustained high temperatures can reduce development rate and survival (Krebs and Feder 1998). In *Manduca*, 1-h heat shocks at 42°C resulted in rapid synthesis of HSPs, and 1-h heat shocks at 38°C increased synthesis of HSPs in some individuals (Fittinghoff and Riddiford 1990). Although high, constant rearing temperatures can increase HSP expression (Karl et al. 2012), the effects of repeated (e.g., daily) exposure to high temperatures are generally unknown. The physiological costs and benefits are likely due to synthesis and levels of HSPs rather than of HSP mRNA levels per se (Feder and Hofmann 1999; Bahrndorff et al. 2009).

Heat shock studies in aquatic and terrestrial ectotherms show responses of heat shock gene expression on timescales of 1–2 h (Feder and Hofmann 1999; Hofmann 1999; Sorensen et al. 2003; Tomanek 2010). In contrast, the timescales of HSP responses vary from hours to days in different insect species (Fittingho and Riddiford 1990; Bahrndorff et al. 2009; Karl et al. 2012). In our simulations, we set $\tau_R = 2$ h and $\tau_P = 20$ h; as a result, the negative effects of HSP on growth rates occur on timescales of a few days, not a few hours (cf. figs. 2*B*, 4*B*–4*D*). Shorter timescales for heat shock expression generate larger fluctuations in growth rates and can increase overall costs to growth (fig. 4; figs. S2, S3 in the supplementary material).

We have emphasized the qualitative predictions of the model, but more quantitative predictions could be obtained by estimating model parameters for a particular study system. For example, repeated measurement of mRNA and protein concentration of, say, Hsp70 following a step increase to a high and constant temperature would allow estimation of the time constants τ_R and τ_P; repeating this experiment at different constant temperatures would allow estimation of the kinetic parameters in equations (2b) and (3b). Standard gravimetric methods could be used to estimate ingestion rates, growth rates, and conversion efficiencies (e.g., Kingsolver and Woods 1997). Quantitative predictions of the parameterized model could then be tested using diurnally fluctuating conditions.

The reduction of growth rate in our model is proportional to protein concentration (fig. 1, eqq. [3]). However, costs of HSPs may be associated with protein synthesis rather than protein concentration per se (Feder and Hofmann 1999; Hofmann 1999). Modeling synthesis and degradation processes separately would allow this to be incorporated into the model. Other mechanisms for feedback from HSPs to growth are also possible. For example, higher HSP concentrations or faster synthesis might directly reduce ingestion rate or conversion efficiency, thereby reducing growth rate (fig. 1). More generally, in the language of systems biology our model represents a proportional-control model, in which negative feedback on growth rate is proportional to some control variable, P. Other types of control are also possible, such as derivative control or integral control, where feedback is related to the rate of change of P (dP/dt) or to P integrated over some time period (Astrom and Murray 2014). Finally, our model assumes that time-dependent costs to growth are due to HSPs, but certainly other genes or gene networks could contribute to such costs. The basic model structure can readily accommodate more complex or system-specific mechanisms of feedback.

Our analyses have emphasized the role played by stress proteins in causing time-dependent costs to growth, but our proposed model can be viewed in a more general context. Suppose that P represents some process that reduces net rates of growth at stressfully high temperatures. For example, P may reflect oxidative or cellular damage or the accumulation of harmful metabolites at higher temperatures. In contrast to our original model (eqq. [1]–[4]), where effects of temperature on P are mediated by changes in gene expression R, suppose $P(T, t)$ is directly influenced by temperature T:

$$\frac{dP}{dt} = -\left[\frac{t}{\tau_P}\right](P - P_f), \tag{6a}$$

$$P_f = \frac{P_m}{\{1 + e^{-a_P(T-T_c)}\}}, \tag{6b}$$

where T_c is the critical midpoint temperature for P and P_m is the maximum level of P. Equations (4) and (6) represent a coupled pair of nonlinear ODEs for dP/dt and dM/dt, which can be solved numerically as before. Results for this simplified model are qualitatively similar to those for the original model: diurnal fluctuations in temperature can produce reductions in mean growth rates over time, with temporal dynamics determined by the timescale of response in P (figs. S4, S5 in the supplementary material). This provides a simple and general framework for understanding time-dependent effects of stress on performance, regardless of mechanistic details. However, the mechanisms underlying time-dependent mechanisms beyond stress proteins warrant further study (Chown and Terblanche 2007; Schulte et al. 2011).

We have focused here on the negative consequences of time-dependent stress responses for growth. Prior exposure to different temperatures can also have positive consequences for ectotherms as a result of physiological acclimation. For example, heat-induced resistance to subsequent heat stress (heat hardening) has been documented in many insects and other ectotherms, and HSPs play an important role in such responses (Hofmann 1999; Hoffmann et al. 2003; Sorensen et al. 2003; Bahrndorff et al. 2009; Karl et al. 2012). Indeed, the adaptive role of HSPs for reducing the negative consequences of brief heat shocks is well established (Lindquist 1986; Lindquist and Craig 1988; Hofmann 1999).

Many other forms of beneficial physiological acclimation to developmental temperature have also been documented (Huey and Bennett 1990; Huey et al. 1999; Angilletta 2009). In *Manduca sexta*, for example, larvae reared in fluctuating temperature conditions had higher maximal growth rates and optimal temperatures than those reared in constant conditions with the same mean temperature (Kingsolver et al. 2015). The mechanisms underlying these acclimation responses are largely unknown, so it is unclear how one might incorporate these effects into models of time-dependent responses. Understanding the mechanisms underlying physiological acclimation beyond heat hardening will be essential for moving beyond thermal performance curves in modeling the consequences of temperature variation and climate change in ectotherms (Schulte et al. 2011).

Acknowledgments

We thank H. MacLean for drafting the figures and R. Huey, M. Kearney, M. Santos, T. Schulte, and L. Sligar for comments on the manuscript. This research was supported in part by National Science Foundation (NSF) grant IOS-1120500 to J.G.K. and by NSF grant IOS-0844916 to H.A.W.

APPENDIX A

Dimensionless Form of the Model

We define the following dimensionless variables (see eqq. [1]–[5]):

$$i = \frac{I}{I_m}, \tag{A1a}$$

$$r = \frac{R}{R_m}, \tag{A1b}$$

$$p = \frac{P}{P_m}, \tag{A1c}$$

$$g = \frac{G}{CI_m}, \tag{A1d}$$

where CI_m represents the maximal growth rate at optimal temperature T_{opt} in the absence of costs of P. Then we can rewrite the model given by equations (1)–(4) in terms of these relative variables. The relative ingestion rate (T) is given by:

$$i(T) = e^{-e[\rho(T-T_{opt})-6]-\sigma(T-T_{opt})^2}. \tag{A2}$$

The relative mRNA concentration $r(T, t)$ depends on both temperature T and time t:

$$\frac{dr}{dt} = -\left[\frac{t}{\tau_R}\right](r - r_f), \tag{A3a}$$

where r_f is defined by

$$r_f = \frac{[R_f/R_m]}{\{1 + e^{-a_R(T-T_c)}\}}. \tag{A3b}$$

The relative protein concentration $p(T, t)$ also depends on both temperature T and time t:

$$\frac{dp}{dt} = -\left[\frac{t}{\tau_P}\right](p - p_f), \tag{A4a}$$

where p_f is given by

$$p_f = \frac{[P_f/P_m]}{\{1 + e^{-a_P(rR_m-R_c)}\}}. \tag{A4b}$$

Relative growth rate, $g(T, t)$, reflects both ingestion rate and the cost of stress proteins. Thus, we have

$$g(T, t) = \frac{dm}{dt} = i(T) - \left[\frac{KP_m}{CI_m}\right]p(T, t). \tag{A5}$$

APPENDIX B

Allometry of Consumption and Growth

The main model does not allow consumption and growth to change as a function of body size. Most organisms, however, eat and grow faster as they grow larger. These effects may be allometric, reflecting that intraspecific (or ontogenetic) increases in metabolic rate often scale allometrically with body size. Allometries can be described by the power law $y = aM^b$, where y is the trait of interest (in our case, consumption and growth), M is body mass, a is the normalization constant, and b is the scaling exponent. In studies of intraspecific scaling, b can vary widely but usually falls between 0.6 and 1.0 (Chown et al. 2007; Killen et al. 2010).

Here we introduce scaling into the model by making ingestion rate depend allometrically on body size. Equation (1) defines ingestion rate as a function of temperature, $I(T)$. We modify this expression to define a scaled ingestion rate:

$$I^*(T) = I(T)aM^b. \tag{B1}$$

When organismal masses rise by several orders of magnitude during growth, we also need to modify the growth equation (eq. [4]) so that costs of P are scaled to body size. We do this using the same approach (and same parameter values):

$$G^*(T, t) = \frac{dM}{dt} = CI^*(T) - KP(T, t)aM^b. \tag{B2}$$

Substituting equation (B1) back into equation (B2) and rearranging gives

$$G^*(T, t) = \frac{dM}{dt} = [CI(T) - KP(T, t)]aM^b. \tag{B3}$$

We used this equation to explore the effects of the ontogenetic scaling exponent, b, on growth trajectories (fig. B1). We set $a = 0.005$. All other parameter values were the same as those used for the analysis in the main text. Not surprisingly, the ontogenetic scaling exponent makes a large difference in overall growth trajectory, especially toward the end of growth over 7 days (168 h).

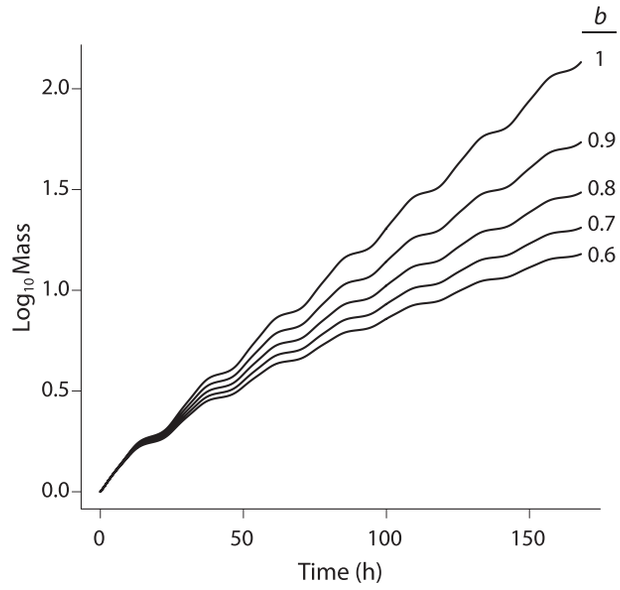


Figure B1: Growth trajectories of larvae calculated from the allometric model. Each of the five lines comes from using a different allometric (ontogenetic) scaling exponent. In all cases, starting mass was set to 1.

Literature Cited Only in Appendix B

Chown, S. L., E. Marais, J. S. Terblanche, C. J. Klok, J. Lighton, and T. M. Blackburn. 2007. Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. Functional Ecology 21:282–290.

Killen, S. S., D. Atkinson, and D. S. Glazier. 2010. The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. Ecology Letters 13:184–193.

Literature Cited

Angilletta, M. J. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, Oxford.

Astrom, K. J., and R. M. Murray. 2014. Feedback systems: an introduction for scientists and engineers. Princeton University Press, Princeton NJ.

Bahrndorff, S., J. Marien, V. Loeschcke, and J. Ellers. 2009. Dynamics of heat-induced thermal stress resistance and Hsp70 expression in the springtail, *Orchesella cincta*. Functional Ecology 23:233–239.

Barshis, D. J., J. T. Ladner, T. A. Oliver, F. O. Seneca, N. Traylor-Knowles, and S. R. Palumbi. 2013. Genomic basis for coral resilience to climate change. Proceedings of the National Academy of Sciences of the USA 110:1387–1392.

Battisti, D. S., and R. L. Naylor. 2009. Historical warnings of future food security with unprecedented seasonal heat. Science 323:240–244.

Chown, S. L., and J. S. Terblanche. 2007. Physiological diversity in insects: ecological and evolutionary contexts. Advances in Insect Physiology 33:50–152.

Dahlhoff, E. P., and N. E. Rank. 2007. The role of stress proteins in responses of a montane willow leaf beetle to environmental temperature variation. Journal of Biosciences 32:477–488.

Deutsch, C. A., J. J. Tewksbury, R. B. Huey, K. S. Sheldon, C. K. Ghalambor, D. C. Haak, and P. R. Martin. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Sciences of the USA 105:6668–6672.

Feder, M. E., and G. E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual Review of Physiology 61:243–282.

Fittinghoff, C. M., and L. M. Riddiford. 1990. Heat sensitivity and protein synthesis during heat-shock in the tobacco hornworm, *Manduca sexta*. Journal of Comparative Physiology B 160:346–356.

Frazier, M., R. B. Huey, and D. Berrigan. 2006. Thermodynamics constrains the evolution of insect population growth rates: "warmer is better." American Naturalist 168:512–520.

Gleason, L. U., and R. S. Burton. 2015. RNA-seq reveals regional differences in transcriptome response to heat stress in the marine snail *Chlorostoma funebralis*. Molecular Ecology 24:610–627.

Hoffmann, A. A., J. G. Sorensen, and V. Loeschcke. 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. Journal of Thermal Biology 28:175–216.

Hofmann, G. E. 1999. Ecologically relevant variation in induction and function of heat shock proteins in marine organisms. American Zoologist 39:889–900.

Huey, R. B., and A. F. Bennett. 1990. Physiological adjustments to fluctuating thermal environments: an ecological and evolutionary perspective. Pages 37–59 *in* R. I. Mormioto, A. Tissières, and C. Georgopoulos, eds. Stress proteins in biology and medicine. Cold Spring Harbor Laboratory Press, New York.

Huey, R. B., D. Berrigan, G. W. Gilchrist, and J. C. Herron. 1999. Testing the adaptive significance of acclimation: a strong inference approach. American Zoologist 39:323–336.

Huey, R. B., C. A. Deutsch, J. J. Tewksbury, L. J. Vitt, P. E. Hertz, H. J. Alvarez Perez, and T. Garland. 2009. Why tropical forest lizards are vulnerable to climate warming. Proceedings of the Royal Society B: Biological Sciences 276:1939–1948.

Huey, R. B., and J. G. Kingsolver. 1989. Evolution of thermal sensitivity of ectotherm performance. Trends in Ecology and Evolution 4:131–135.

Huey, R. B., and R. D. Stevenson. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. American Zoologist 19:357–366.

Intergovernmental Panel on Climate Change. 2007. Climate change 2007: the physical science basis. Cambridge University Press, Cambridge.

Karl, I., C. Michalowsky, J. G. Sorensen, V. Loeschcke, and K. Fischer. 2012. Effects of rearing and induction temperature on the temporal dynamics of heat shock protein 70 expression in a butterfly. Physiological Entomology 37:103–108.

Kingsolver, J. G. 2000. Feeding, growth and the thermal environment of cabbage white caterpillars, *Pieris rapae* L. Physiological and Biochemical Zoology 73:621–628.

Kingsolver, J. G., S. E. Diamond, and L. B. Buckley. 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. Functional Ecology 27:1415–1423.

Kingsolver, J. G., J. K. Higgins, and K. Augustine. 2015. Fluctuating temperatures and ectotherm growth: distinguishing non-linear and time-dependent effects. Journal of Experimental Biology 218:2218–2225.

Kingsolver, J. G., and A. M. Nagle. 2007. Evolutionary divergence in thermal sensitivity and diapause of field and laboratory populations of *Manduca sexta*. Physiological and Biochemical Zoology 80:473–479.

Kingsolver, J. G., G. J. Ragland, and S. E. Diamond. 2009. Evolution in the constant environment: thermal fluctuations and thermal sensitivity in laboratory and field populations of *Manduca sexta*. Evolution 63:537–541.

Kingsolver, J. G., G. J. Ragland, and J. G. Shlichta. 2004. Quantitative genetics of continuous reaction norms: thermal sensitivity of caterpillar growth rates. Evolution 58:1521–1529.

Kingsolver, J. G., and H. A. Woods. 1997. Thermal sensitivity of growth and feeding in *Manduca sexta* caterpillars. Physiological Zoology 70:631–638.

———. 2016. Data from: Beyond thermal performance curves: modeling time-dependent effects of thermal stress on ectotherm growth rates. American Naturalist, Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.1017n.

Krebs, R. A., and M. E. Feder. 1998. Hsp70 and larval thermotolerance in *Drosophila melanogaster*: how much is enough and when is more too much? Journal of Insect Physiology 44:1091–1101.

Kregel, K. C. 2002. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. Journal of Applied Physiology 92:2177–2186.

Lindquist, S. 1986. The heat-shock response. Annual Review of Biochemistry 55:1151–1191.

Lindquist, S., and E. A. Craig. 1988. The heat shock proteins. Annual Review of Genetics 22:631–677.

Martin, T. L., and R. B. Huey. 2008. Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. American Naturalist 171:E102–E118.

Meyer, E., G. V. Aglyamova, and M. V. Matz. 2011. Profiling gene expression responses of coral larvae (*Acropora millepora*) to elevated temperature and settlement inducers using a novel RNA-seq procedure. Molecular Ecology 20:3599–3616.

Mislan, K. A. S., B. Helmuth, and D. S. Wethey. 2014. Geographical variation in climatic sensitivity of intertidal mussel zonation. Global Ecology and Biogeography 23:744–756.

Niehaus, A. C., M. J. Angilletta, M. W. Sears, C. E. Franklin, and R. S. Wilson. 2012. Predicting the physiological performance of ectotherms in fluctuations thermal environments. Journal of Experimental Biology 215:694–701.

Petersen, C., H. A. Woods, and J. G. Kingsolver. 2000. Stage-specific effects of temperature and dietary protein on growth and survival of *Manduca sexta* caterpillars. Physiological Entomology 25:35–40.

Porcelli, D., R. K. Butlin, K. J. Gaston, D. Joly, and R. R. Snook. 2015. The environmental genomics of metazoan thermal adaptation. Heredity 114:502–514.

Potter, K., G. Davidowitz, and H. A. Woods. 2009. Insect eggs protected from high temperature by limited homeothermy of plant leaves. Journal of Experimental Biology 212:3448–3454.

Reynolds, S. E., and S. F. Nottingham. 1985. Effects of temperature on growth and efficiency of food utilization in fifth instar caterpillars of the tobacco hornworm, *Manduca sexta*. Journal of Insect Physiology 31:129–134.

Rezende, E. L., L. E. Castaneda, and M. Santos. 2014. Tolerance landscapes in thermal ecology. Functional Ecology 28:799–809.

Schoville, S. D., F. S. Barreto, G. W. Moy, A. Wolff, and R. S. Burton. 2012. Investigating the molecular basis of local adaptation to thermal stress: population differences in gene expression across the transcriptome of the copepod *Tigriopus californicus*. BMC Evolutionary Biology 12:170.

Schulte, P. M., T. M. Healy, and N. A. Fangue. 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integrative and Comparative Biology 51:691–702.

Seneca, F. O., and S. R. Palumbi. 2015. The role of transcriptome resilience in resistance of corals to bleaching. Molecular Ecology 24:1467–1484.

Sinervo, B., F. Méndez-de-la-Cruz, D. B. Miles, B. Heulin, E. Bastiaans, M. Villagrán-Santa Cruz, R. Lara-Resendiz, et al. 2010. Erosion of lizard diversity by climate change and altered thermal niches. Science 328:894–899.

Smith, S., L. Bernatchez, and L. B. Beheregaray. 2013. RNA-seq analysis reveals extensive transcriptional plasticity to temperature stress in a freshwater fish species. BMC Genomics 14:375.

Soetaert, K., T. Petzoldt, and R. W. Setzer. 2010. Solving differential equations in R: package deSolve. Journal of Statistical Software 33:1–25.

Sorensen, J. G., T. N. Kristensen, and V. Loeschcke. 2003. The evolutionary and ecological role of heat-shock proteins. Ecology Letters 6:1025–1037.

Sorensen, J. G., M. M. Nielsen, M. Kruhoffer, J. Justesen, and V. Loeschcke. 2005. Full genome gene expression analysis of the heat stress response, in *Drosophila melanogaster*. Cell Stress and Chaperones 10:312–328.

Sunday, J. M., A. E. Bates, and N. K. Dulvy. 2011. Global analysis of thermal tolerance and latitude in ectotherms. Proceedings of the Royal Society B: Biological Sciences 278:1823–1830.

Tomanek, L. 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographic distribution ranges and metabolic costs. Journal of Experimental Biology 213:971–979.

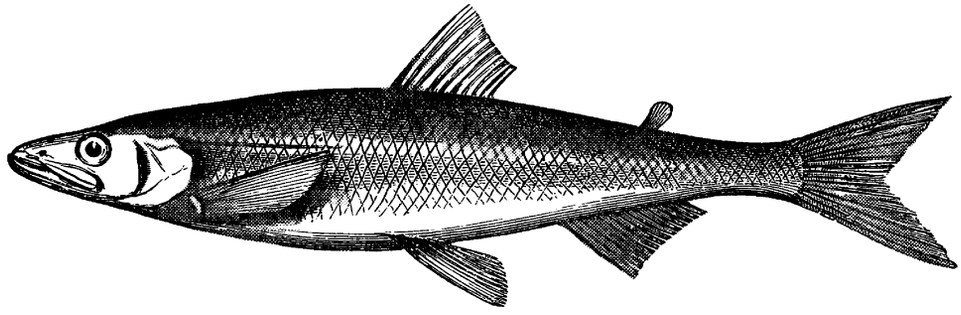
Vasseur, D. A., J. P. DeLong, B. Gilbert, H. S. Greig, C. D. Harley, K. S. McCann, V. M. Savage, T. D. Tunney, and M. I. O'Connor. 2014. Increased temperature variation poses a greater risk to species than climate warming. Proceedings of the Royal Society B: Biological Sciences 281:2612–2620.

Williams, J. W., and S. T. Jackson. 2007. Novel climates, no-analog communities, and ecological surprises. Frontiers in Ecology and the Environment 5:475–485.

Williams, J. W., S. T. Jackson, and J. E. Kutzbach. 2007. Projected distributions of novel and disappearing climates by 2100 AD. Proceedings of the National Academy of Sciences of the USA 104:5738–5742.

Woods, H. A., and R. T. Bonnecaze. 2006. Insect eggs at a transition between diffusion and reaction limitation: temperature, oxygen, and water. Journal of Theoretical Biology 243:483–492.

Associate Editor: Michael Kearney
Editor: Yannis Michalakis



"Frost-fish (*Osmerus mordax*). We desire to record here the fact of the presence of this fish in a few numbers during almost every month of the year. In August when the young shad are going down the river, we have seen single specimens of 'smelt,' or 'frost-fish,' as they are generally called." From "Notes on Fresh-Water Fishes of New Jersey" by Charles C. Abbott (*The American Naturalist*, 1870, 4:99–117).